The Influence of Benomyl Formulation on the Response of Cucumber Seedlings (*Cucumis sativus*) to Dibutylurea

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Abstract: Experiments were conducted in a glasshouse to evaluate the phytotoxicity of dibutylurea (DBU), a breakdown product of benomyl, benomyl formulations and constitutive ingredients of 'Benlate' Dry Flowable (DF) on whole cucumber (*Cucumis sativus*. Poinsett 76) plants. Benlate Wettable Powder (WP) and DF formulations did not affect the phytotoxicity of DBU. When commercially purchased (samples obtained from growers) DF and WP formulations containing equivalent DBU concentrations were compared for phytotoxicity, no statistical interactions between formulations were observed. Benomyl, DBU and DF inert ingredients were tested alone and in combination to assess their phytotoxicity. Only DBU at rates greater than 94 mg litre^{-1} caused a reduction in root and shoot growth. Also DBU applied alone or as a component of either Benlate formulation was shown to reduce the peak-to-terminal chlorophyll *a* fluorescence ratio measured *in vivo* by a plant productivity fluorometer. © 1998 SCI.

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1 INTRODUCTION

Benomyl [methyl 1-(butylcarbamoyl)benzimidazole-2-ylcarbamate] is the active ingredient in Benlate (E. I. du Pont de Nemours and Co. (Inc.), Agricultural Products Dept, Wilmington, DE) fungicide formulations, and has been widely used for disease control and as a miticide. Benlate 50WP (benomyl 500 g kg^{-1} wettable powder) has been in use since 1969. Benomyl was introduced as a 500 g kg^{-1} active ingredient dry flowable (water dispensable granule, WG), Benlate 50DF in 1987. During the 25-year history of benomyl use, only occasional instances of phytotoxicity have been reported.[1–3] More recently, however, claims of crop damage from Benlate DF have been reported,[4] but little or no research has been published to corroborate these reports.

Moye *et al.*[5] examined 56 samples of benomyl formulations collected from various sources and found *N*,*N*′-dibutylurea (DBU), in concentrations ranging from 0·10 to 8·85%, present in both WP and DF formulations. Benomyl in water rapidly degrades to form methyl-2-benzimidazole carbamate (MBC) and *n*-butyl isocyanate (BIC).[6] Moye *et al.*[5] demonstrated that BIC forms DBU in water, soil and on plant surfaces. Tolson and Moye[7] demonstrated that the degradation of benomyl to form DBU is dependent on temperature and especially on humidity in the storage environment, and that, at low humidity, DBU forms more readily in the DF formulation than in the WP formulation. Shilling *et al.*[8] reported that DBU is toxic to cucumber seedlings as a soil

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drench at rates of 2·8 kg ha^{-1} and higher. These findings, however, do not fully explain why recent reports of plant injury have been attributed primarily to the DF formulation rather than the WP formulation. In addition, the role of DBU in the plant damage claimed to have been caused by benomyl under field conditions has not been studied.

The objectives of this study were: (1) to assess the phytotoxic effects of single-drench benomyl applications with varying DBU concentrations on cucumber seedlings; (2) to determine if equivalent DBU-containing lots of Benlate DF are more phytotoxic than Benlate WP formulations; (3) to determine potential interactive effects between DBU, benomyl and the inert ingredients in Benlate DF formulations; and (4) to assess the relative effects of Benlate WP and DF with known concentrations of DBU on chlorophyll *a* fluorescence.

2 MATERIALS AND METHODS

In all studies the treatments were applied as a soil drench in 935 litre ha^{-1} diluent volume, equivalent to 55 ml of 1·20 mg ml^{-1} benomyl dispersion in each pot (the 1990 label rate for benomyl applications to container plants),[9] to 10-day-old cucumber seedlings. Cucumbers were seeded four per pot into 650-cm^3 pots filled with commercial potting soil and thinned to two plants per pot after emergence. Plants were maintained in a greenhouse under the following conditions: 15 : 9 h light : dark photoperiod, average light intensity at noon of 1500 μM m^{-2} s^{-1}, and average mean temperature of 29 $\pm$ 4°C. Overall visual appearance of the plants was noted throughout the experiments. Shoot and root biomass were measured two weeks after treatment. Shoots were cut at the soil surface, placed in paper bags, and oven-dried at 60°C for 48 h. Roots were gently washed free of soil and likewise bagged and dried.

The Benlate DF inert ingredients mixture (Confidential Statement of Formulation was released by E. I. du Pont de Nemours and Co. (Inc.), Agricultural Products Dept, Wilmington, DE) was prepared from sucrose 222, sodium lignosulfonate 111, sodium dodecylbenzene sulfonate 44, polyvinyl pyrrolidone 44, silica (Aldrich Chemical Co.) 22 and powdered redried starch A (A. E. Staley Co.) 555 g kg^{-1}. These materials were mixed with a mortar and pestle until ground to a powdery consistency.

A randomized complete block design was used for all experiments and analyses of variance were performed. All experiments were conducted twice and treatments replicated four times. Because there were no experiment-by-treatment interactions ($P > 0{\cdot}05$), data were combined and treatment means compared to the untreated control mean using Dunnett's Test. Means followed by standard errors from both experiments within each study are presented. Four separate studies were conducted.

2.1 Fortification study

A fortification study compared Benlate 50DF and Benlate 50WP, each fortified with DBU (ICN, 99%) and compared to DBU alone at the same concentrations. Selected lot numbers of Benlate 50DF (DFD) and Benlate 50WP (WPA) (Table 1) each with a DBU concentration of 0·45%, were prepared at label drench rates and fortified with DBU to achieve concentrations of 0·44, 14·4, 50·0, 100·0 and 250·0 mg litre^{-1} DBU. Each DBU-fortified formulation and a water blank used for the control were applied as previously described.

2.2 DBU content study

A second study evaluated phytotoxicity of commercially purchased Benlate 50WP and Benlate 50DF formulations with low (approximately 0·44% by weight), medium (approximately 3·5% by weight), and high (approximately 7·0% by weight) contents of DBU. When prepared and applied at label drench rates the DBU concentrations were 6·0, 42·0 and 84·0 mg litre^{-1} DBU. These treatments were compared to the same concentrations of DBU alone.

2.3 Interaction of formulation and DBU study

The third study evaluated interactions of the inert ingredients in the Benlate 50DF formulation with DBU and benomyl. Two concentrations each of benomyl, Benlate DF inert ingredients, and DBU, along with the representative controls, were applied alone and in all possible combinations to cucumbers. The 47 and 94 mg litre^{-1} DBU concentrations were equivalent to single-drench Benlate DF applications containing 3·92 and 7·84%

TABLE 1
Summary of Benlate Formulations used and Respective DBU Concentrations Naturally Present

Lot no.	*Sample*[a]	*DBU*[b] *(% by wt)*
UO73190-738	DFD	0·44
U83189	DF5065	3·49
2498	DF4	6·71
F042391E	WPA	0·45
EP42?61MS[c]	WPB	3·44
F60505H	WPE	7·52

[a] DF, dry flowable formulation; WP, wettable powder formulation.
[b] DBU content determined by the methods of Moye *et al.*[4]
[c] Lot number partially illegible.

DBU, respectively. The selection of dosage regimes for benomyl and inert ingredients was based on the amount of product used in a single-drench application of Benlate DF. Benomyl content in Benlate DF represents 55% by weight of product with the remaining amount made up of inert ingredients. The 550 and 1100 mg litre^{-1} concentrations of benomyl were equivalent to 1× and 2× single dose rate, respectively, of 1·20 mg litre^{-1} as specified on the product label. Inert ingredient rates of 360 and 1800 mg litre^{-1} were equivalent to 0·5× and 3× label rates.

2.4 Chlorophyll *a* fluorescence study

Substituted ureas herbicides exert their phytotoxic effects by inhibiting the electron transport chain in photosystem II.[10] Chlorophyll *a* fluorescence, a measure of electron transport chain activity, was measured *in vivo* from whole plants that had been treated with diuron, Benlate 50DF, Benlate 50WP or DBU. Benlate 50WP (Sample WPE) and Benlate 50DF (Sample DF4), which were used in the previous experiments (Table 1), were applied as single-dose soil drenches to 10-day-old cucumber seedlings at appropriate concentrations to achieve 7% DBU (equal to 84·0 mg litre^{-1} DBU). These lots of Benlate WP and Benlate DF were selected because they were approximately equal in DBU concentration. Likewise, a control 7% DBU concentration was applied alone. Diuron, a known electron-transport inhibitor, was soil-applied at a concentration of 0·23 mg litre^{-1}. Chlorophyll *a* fluorescence in whole plants was then measured for 50 s (Model SF-20 plant productivity fluorometer, Richard Brancker Research Ltd, Ottawa, Canada). The parameters of the fluorescence induction curve, initial (I), peak (P) and terminal (T) fluorescence, were recorded. An excitation wavelength of 670 nm was used, and fluorescence was measured at 710 nm and above. Fluorescence measurements were taken at 1, 2, 4 and 24 h after treatment. Fluorescence (F: peak-to-terminal ratio) is expressed as a ratio using the formula: $F = (P - I)/(T - I)$.

3 RESULTS AND DISCUSSION

3.1 Fortification study

Benomyl formulation fortified with DBU was not more phytotoxic than DBU alone, and no formulation × concentration interaction existed. DBU, regardless of formulation, decreased shoot and root biomass at the highest concentration (Table 2). Shoot biomass was reduced 50 and 53% by the DFD and WPA formulations, respectively, at the 250 mg litre^{-1} DBU concentration, compared to a 62% reduction at the same

TABLE 2
Effect of DF and WP Benlate Formulations Fortified with DBU and DBU Alone on Shoot Weight of 24-Day-Old Cucumber Seedlings 14 Days after Treatment

Formulation	*DBU concentration (mg litre^{-1})*	*Shoot weight[a] (g plant^{-1})*	*Root weight[a] (g plant^{-1})*
DFD	Control	2·46	0·62
	0·44	2·43	0·64
	14·4	1·94	0·56
	50·0	2·58	0·71
	100·0	2·46	0·54
	250·0	1·22*	0·25*
WPA	Control	2·52	0·61
	0·44	2·15	0·57
	14·4	2·22	0·62
	50·0	2·45	0·56
	100·0	1·78	0·43
	250·0	1·18*	0·19*
DBU	Control	2·55	0·60
	0·44	2·42	0·54
	14·4	2·61	0·56
	50·0	2·64	0·61
	100·0	2·08	0·48
	250·0	0·96*	0·16*

[a] Mean values followed by an asterisk within columns are significantly different from the untreated control based on Dunnett's Test, $P < 0{\cdot}05$.

concentration of DBU alone (Table 2). Root biomass was reduced 60 and 69% by the fortified DF and WP formulations, respectively, at the 250 mg litre^{-1} DBU concentration, compared to a 73% reduction at the same concentration of DBU alone (Table 2).

3.2 DBU content study

The type of formulation did not influence shoot and root weight when formulations with naturally occurring DBU concentrations were applied. Neither shoot nor root biomass was significantly reduced by the benomyl formulations or DBU at concentrations up to 84·0 mg litre^{-1} (the maximum applied in this study; data not shown).

3.3 Interaction of formulation and DBU study

Of the three compounds evaluated—benomyl, the inert ingredients, and DBU—only DBU reduced shoot and root growth. DBU decreased shoot and root biomass by 32 and 53%, respectively, at 94 mg litre^{-1} (Table 3). Although comparable amounts of DBU in the fortification study (Table 2) produced decreased root and shoot weight the effects did not reach statistical significance until the 250 mg litre^{-1} DBU concentration. Variation

TABLE 3
Effect of Benomyl, Inert Ingredients and DBU on Shoot Growth of 24-Day-Old Cucumber Seedlings 14 Days after Treatment

Formulation	*Concentration* (*mg litre*$^{-1}$)	*Shoot weight*[a] (*g plant*$^{-1}$)	*Root weight*[a] (*g plant*$^{-1}$)
Benomyl	Control	2·17	0·38
	550	2·18	0·40
	1100	2·31	0·42
Inerts	Control	2·23	0·40
	360	2·25	0·40
	1800	2·17	0·39
DBU	Control	2·62	0·53
	47	2·22	0·41
	94	1·81*	0·25*

[a] Mean values followed by an asterisk within columns are significantly different from the untreated control based on Dunnett's Test, $P < 0{\cdot}05$.

in response between these experiments may be attributed to experiment-to-experiment fluctuation in light intensity which may affect the transpiration rate and thus the kinetics of the adsorbed DBU dose.

3.4 Chlorophyll *a* fluorescence study

Diuron, DBU and DBU-containing Benlate DF4 and WPE formulation affected the chlorophyll *a* fluorescence by cucumber, reducing the peak-to-terminal fluorescence ratio relative to the control (Table 4). DBU applied alone significantly reduced the fluorescence 2 to 24 h later. The benomyl formulations containing DBU reduced fluorescence in a similar manner statistically significant even after 1 h application. The magnitude of reduction in peak-to-terminal ratio caused by diuron was similar to that caused by the benomyl formulations tested. However, the drop in the peak-to-terminal ratio caused by DBU alone was slower to develop than the drop caused by the DF and WP formulations. The formulations probably increased the solubility and uptake of the chemical, allowing the DBU to reach the site-of-action more quickly. Benlate 50DF and Benlate 50WP were equal in phytotoxicity when they contained equal amounts of DBU. Growth inhibition in 10-day-old cucumber seedlings was dependent only on the amount of DBU present for both DBU-fortified formulations and formulations with naturally occurring DBU. DBU was determined to be responsible for growth inhibition when applied alone or in combustion with benomyl and the inert ingredients of the Benlate DF formulation. Because no meaningful biological interactions were observed among these three components, an interaction between DBU and benomyl formulation is unlikely. Likewise, the reductions in chlorophyll *a* fluorescence peak-to-terminal ratio caused by DBU, Benlate 50DF and Benlate 50WP were very similar, and depended only on DBU concentration. Finally, the injury symptoms to cucumber seedlings were nearly identical in all studies and for all formulations and DBU alone. These symptoms were also similar to those reported in previous studies, and consisted of interveinal and leaf marginal chlorosis, downward cupping of leaf margins and stunting.[8] Based on these studies, using the Benlate formulations and lot numbers described, the phytotoxicity produced by a DBU-contaminated benomyl drench was quantitatively and qualitatively the same as that of DBU alone.

TABLE 4
Effect of Diuron, Benlate 50DF, Benlate 50WP and DBU on the Peak-to-Terminal Ratio of Chlorophyll *a* Fluorescence by Cucumber Seedlings

Treatment	*Time after treatment*[a]			
	1 h	*2 h*	*4 h*	*24 h*
Control	2·76 a	2·69 a	2·62 a	2·92 a
Diuron[b]	1·82 a	1·61 b	1·09 b	0·91 b
Benlate DF4[b]	1·74 b	1·65 b	1·13 b	1·16 b
Benlate WPE[b]	1·82 b	1·67 b	1·24 b	1·22 b
DBU[b]	2·09 a	1·97 b	1·36 b	1·23 b

[a] Mean values followed by different letters within columns are significantly different based on Duncan's Multiple Range Test, $P < 0{\cdot}05$.
[b] Concentration of diuron was 0·23 mg litre^{-1}; adjusted concentration of DBU in Benlate DF4 and WPE (see Table 1) application was 84 mg litre^{-1}; concentration of DBU application was 84 mg litre^{-1}.

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